

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Gregory H. Altman
Application No.: 10/800,134 Art Unit: 1657
Filed: March 11, 2004 Examiner: Naff, David M.
Confirmation No.: 6963
For: IMMUNONEUTRAL SILK-FIBER-BASED MEDICAL DEVICES

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF THE INVENTORS, UNDER 37 C.F.R. § 1.131

We, Gregory H. Altman, David L. Kaplan, Rebecca L. Horan and David J. Horan, hereby declare as follows:

1. We are the inventors of the above-described patent application.
2. We have been advised that the Examiner has cited a U.S. Patent No. 7,285,637 to Armato et al. ("Armato"), filed on September 28, 2001, in connection with the examination of the above-identified patent application.
3. Prior to September 28, 2001 we had conceived of and reduced to practice, in the United States, the invention as claimed in the above captioned U.S. Patent Application Ser. No. 10/800,134 as evidenced by the attached email document and the abstract document which was sent as an attachment to the aforementioned email. The email and abstract are attached herewith as Exhibits A and B, respectively. Confidential information has been redacted. The date of the Exhibits is prior to September 28, 2001.

4. Exhibit A describes the new and novel silk matrix as designed to have similar mechanical properties to the anterior cruciate ligament. Although the date has been redacted on this email, we confirm that the email is dated prior to September 28, 2001.

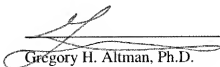
5. Exhibit B describes an as-claimed sericin-extracted silkworm fibroin fiber construct. More specifically, the first page of Exhibit B, fourth full paragraph, describes a "six-cord construct" in which fibers are bundled and twisted into "strands" and then twisted into "cords" which are arranged in parallel to form a prosthetic ACL. Although the terms "fabric" or "yarn" are not mentioned, per se, the several of the silk fiber structures in this paragraph are a "yarn" or "fabric" of the claimed invention. Although the date has been redacted on this abstract, we confirm that the abstract is dated prior to September 28, 2001.

6. Accordingly, Armato et al. teaches no more than what we had already accomplished in the United States prior to its publication date.

7. We diligently continued our laboratory work, in the United States, until the date of filing our patent application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent that issues therefrom.

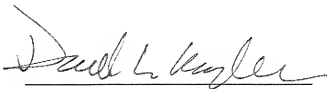
4-10-09
Date


Gregory H. Altman, Ph.D.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent that issues therefrom.

15 April 2009

Date



David L. Kaplan, Ph.D.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent that issues therefrom.

10 APR 2009

Date



Rebecca L. Horan, Ph.D.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent that issues therefrom.

14 APRIL 2009

Date

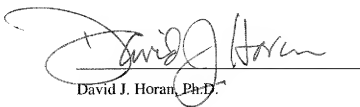

David J. Horan, Ph.D.

Exhibit A

Altman To 01/68

Subject: Re: Status Update

Date: [REDACTED]

From: Greg Altman <galtman@emerald.tufts.edu>

Organization: Tufts University

To: "Morency, Michel" <MMorency@Mintz.com>, Martin Son <martin.son@tufts.edu>

CC: Mo Glynn <mglynn@eecs.tufts.edu>

Hi Mike,


Redacted

Matrix. A new and novel silk matrix (fibers organized in a wire rope design) was designed and tested independent of Tufts. The matrix was designed to have similar mechanical properties to the anterior cruciate ligament. [REDACTED]

[REDACTED] Some matrix verification was performed at Tufts in combination with our bioreactor.

Redacted

Redacted

 Matrix Abstract [REDACTED].doc	<p>Name: Matrix Abstract [REDACTED].doc Type: Winword File (application/msword) Encoding: base64 Download Status: Not downloaded with message</p>
---	---

Introduction

The high incidence of anterior cruciate ligament (ACL) failures in the United States, coupled with the absence of adequate options to restore full knee joint function, have prompted the development of new tissue engineering strategies. ACL ruptures can result in severe limitations in mobility, pain and discomfort, and inability to participate in sports and exercise. The ACL has poor healing capabilities, and no surgical procedure has been shown to completely and adequately restore knee function without associated side effects, e.g., long recovery periods, muscle atrophy, tendonitis, arthritis.

Tissue engineering can potentially provide improved clinical options in orthopaedic medicine through the *in vitro* generation of biologically based functional tissues for transplantation at the time of injury or disease. Further, adult stem cells are becoming increasingly recognized for their potential to generate different cell types and thereby function effectively *in vitro* or *in vivo* in tissue repair¹. The knee joint geometry and kinematics and the resultant effects on ACL structure must be incorporated into construct design if a tissue engineered ACL generated *in vitro* is to successfully stabilize the knee and function *in vivo*. A mismatch in the ACL structure-function relationship would result in graft failure.

The purpose of this project is to engineer mechanically and biologically functional autologous ACL using a novel silk-fiber-based matrix, bone marrow stromal cells and a complex 3D bioreactor environment to induce *de novo* ligament tissue formation *in vitro* prior to implantation. This study focuses on the development of the silk-based matrix with mechanically relevant properties and the initial evaluation of adult progenitor stem cell attachment and proliferation on the ligament matrix.

Materials & Methods

Costello's equation⁴ for a three-strand wire rope was derived to predict mechanical properties of a novel twisted silk-based matrix. Raw *Bombyx Mori* silkworm fibers (Fig. 1a) were washed as previously described¹ to remove sericin, the glue-like protein coating the native silk fibroin (Fig. 1b). Mechanical properties of the silk fibroin were characterized and an applicable geometry was derived for more detailed analysis. A six-cord construct was chosen for use as an ACL replacement. Following is the geometrical hierarchy of our construct: 1 ACL prosthesis = 6 parallel cords; 1 cord = 3 twisted strands (3 twists/cm); 1 stand = 6 twisted bundles (3 twists/cm); 1 bundle = 30 parallel washed fibers. The number of fibers and geometry were selected such that the silk prosthesis¹ is similar to the ACL biomechanical properties in UTS, linear stiffness, yield point and % elongation at break⁵ (Table 1), serving as a solid starting point for the development of a tissue engineered ACL.

Mechanical Analysis was performed using a servohydraulic Instron 8511 tension/compression system with Fast-Track software. Single pull-to-failure and fatigue analysis were performed on single cords. Data was extrapolated to represent a 6-cord ACL prosthesis (Fig. 2). Cord ends were embedded in an epoxy mold to generate a 3 cm long construct between anchors. Single pull to failure testing was performed at a strain rate of 100%/sec and data analyzed using Instron Series IX software. Cycles to failure at UTS, 1,680N, and 1,200N ($n=5$ for each load) were determined using a H-sine wave function at 1Hz generated by Wavemaker32 ver. 6.6 (Instron, MA). Fatigue testing was conducted in a neutral phosphate buffered saline (PBS) solution at room temperature.

Human BMSCs were isolated as previously described³ and culture expanded to the 2nd passage in cell culture medium with 10% FBS, supplemented with amino acids, antibiotics, and 1 ng/ml basic fibroblast growth factor. Individual silk cords, 2 cm in length, were fixed in 6-well tissue culture plates and EtO sterilized. BMSCs were seeded at a density of 70,000 cells/cord and allowed to attach on the fiber bundle for 30 min, after which 2 ml of supplemented DMEM were added to each well. Seeded matrices were cultured for 1, 7, 14 and 21 days. Cell attachment and spreading were monitored using SEM and proliferation measured by a fluorimetric assay using the PicoGreen dsDNA Quantitation kit.

Results

Removal of sericin from silk fibers altered the ultrastructure of the fibers, resulting in a smoother fiber surface and the underlying silk fibroin was revealed (Fig. 1b), with average diameter ranging between 20-40 μ m. The mechanical properties of the optimized silk matrix are summarized in Table 1. It is evident from these results that the optimized silk matrix exhibited values comparable to those of native ACL, which was reported² to have an average UTS of ~2100 N, stiffness of ~250 N/mm, yield point of ~1200 N, and 33% elongation at break. Regression analysis of matrix fatigue data (Fig. 2), when extrapolated to physiological load levels⁶ (400 N) to predicate number of cycles to failure *in vivo*, indicates a matrix life of 3.3 million cycles. It is clear that the wire rope matrix design utilizing washed silk fibers resulted in a matrix with physiologically equivalent structural properties, and it is a suitable scaffold for ligament tissue engineering.

UTS (N)	Stiffness (N/mm)	Yield Pt. (N)	Elongation (%)
2137 \pm 72	254 \pm 26	1262 \pm 36	38.6 \pm 2.4

Table 1. Mechanical properties of novel silk matrix, $n=5$.

The response of bone marrow stromal cells to the silk matrix was also examined. BMSCs readily attached and grew on the silk matrix after 1 day in culture (Fig. 1c), and formed cellular extensions to bridge neighboring fibers. A uniform cell sheet covering the construct was observed at 21 days of culture (Fig. 1d). Total DNA quantification confirmed that BMSCs proliferated and grew on the silk construct with the highest amount of DNA measured after 21 days in culture.

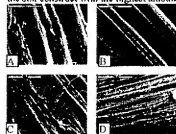


Figure 1.

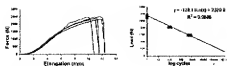


Figure 2. Load-elongation and fatigue histograms.

Conclusions

The novel silk wire rope based matrix possesses mechanical properties matching those of native human ACL, and was able to support the attachment and proliferation of BMSCs. Future work will focus on *in vitro* differentiation and the effect of mechanical regiment on BMSC response on silk matrix in a bioreactor system.

1. Sefin, S., *et al.* *J. Biomedical Materials Research*, 54:139-148 (2000).
2. Woo, SL-Y., D. J. Adams *Knee Ligaments: Structure, Function, Injury and Repair*, Ed. D Daniel *et al.* Raven Press 1990.
3. Sussman, M., *Nature* 410, 640 (2001)
4. Costello, G. A., *Theory of Wire Rope* Springer 1997
5. Brader *et al.* *J. Bone Joint Surgery*, 1998.
6. Bolton, W., *et al.*, *Clinical Orthopedics*, 1985